# Interaction of Fibronectin with Collagen Fibrils†

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ABSTRACT: The interaction of fibronectin with native collagen during collagen fibril formation was investigated. Fibronectin prepared from serum, or from the cell surface, bound to the forming collagen fibrils while less fibronectin bound to preformed fibers. Denatured collagen competed with native collagen in binding fibronectin. Fibronectin delayed the precipitation of collagen fibrils but did not alter the total amount of fibrils formed. Fibronectin which was heated to 80 °C for 30 min did not promote cell adhesion but still bound to native collagen and delayed fiber formation. The collagen-binding fragment of fibronectin produced by digestion either with chymotrypsin or with neutrophil elastase had a similar effect in delaying fibril formation, but the cell-binding fragment was not active. These studies indicate that fibronectin can bind to aggregating collagen fibers probably at the same site shown previously to bind to denatured collagen. Since fibronectin inhibits the rate of collagen fibrillogenesis, it may regulate the size of collagen fibers.

Fibronectin is a large glycoprotein found on the surface of cells, in the extracellular matrix, and in blood (Yamada & Olden, 1978; Vaheri & Mosher, 1978; Pearlstein et al., 1980). It has been shown that fibronectin promotes the attachment of fibroblastic cells to collagen substrates in vitro (Klebe, 1974; Pearlstein, 1976). The binding of fibronectin to collagen probably causes a conformational change which allows fibronectin to bind to the cell surface (Pearlstein, 1977). Fibronectin binds strongly and specifically to denatured collagen (Kleinman et al., 1976, 1978; Hopper et al., 1976; Engvall & Ruoslahti, 1977; Jilek & Hörmann, 1978; Engvall et al., 1978), and denatured collagen affinity columns are used routinely to isolate fibronectin from serum, culture media, and tissue extracts. Relatively strong chaotropic agents such as 1 M KBr (Hopper et al., 1976), 8 M urea (Engvall & Ruoslahti, 1977), or 1 M arginine (Vuento & Vaheri, 1978) are needed to elute the fibronectin from the collagen.

It is known that the major binding site for fibronectin in the $\alpha$1(I) chain of collagen is located between residues 693 and 795 (Kleinman et al., 1978; Dessau et al., 1978). This region is unique since it contains the peptide bond cleaved by animal collagenases (residues 775–776). Cleavage of this bond (775–776) or the bond between residues 779 and 780 destroys the binding activity for fibronectin (Kleinman et al., 1978). A stretch of four triplets in this region lacks hydroxyproline and proline and contains hydrophobic amino acids, and thus may form a labile triple helix (Highberger et al., 1978).

The nature of the interaction between fibronectin and native collagen is less well understood. Studies in vitro indicate that native collagen binds less fibronectin than does denatured collagen (Kleinman et al., 1976; Dessau et al., 1978; Jilek & Hörmann, 1978, Engvall et al., 1978). Fibronectin does bind to native collagen, and the rate of binding is much faster in the presence of heparin (Johansson & Höök, 1980). Immunocytochemical studies have shown that collagen and fibronectin are codistributed in tissues (Bornstein & Ash, 1977; Vaheri et al., 1978; Hedman et al., 1979). Furthermore, fibronectin is observed in a regularly distributed array along collagen fibers synthesized by cells in culture (Furcht et al., 1980).

It is possible that the difficulty in demonstrating an interaction between fibronectin and collagen has been due to the conditions used in studying the interaction. For example, binding sites on collagen molecules which are located in the internal portions of collagen fibrils would not be accessible. Alternatively, when the binding of fibronectin to native collagen is examined at low temperatures, the sequence of amino acids constituting the binding site might be helical and thus not reactive with fibronectin. Since the same cell secretes both collagen and fibronectin, we thought that the interaction between the two molecules might be most readily detected with monomers or small aggregates of collagen under physiological conditions of temperature, pH, and ionic strength. Under these conditions, collagen molecules self-assemble into fibrils. Our studies show that fibronectin binds to collagen fibrils under these conditions and that fibronectin alters the kinetics of fibril formation.

## Materials and Methods

*Collagen Preparations.* Rat tail tendon collagen was prepared from an acid extract of tail tendon, purified by standard procedures, and stored after lyophilization (Linsenmayer et al., 1978). Weighed amounts of the lyophilized collagen were dissolved at 4 mg/mL in 0.5 N acetic acid and stored in small aliquots at −20 °C. Collagen from the skins of lathyritic rats was prepared as previously described (Bornstein & Piez, 1966). After denaturation, collagen was cleaved with cyanogen bromide, and various peptides were isolated by carboxymethylcellulose column chromatography. *Ascaris* collagen was a gift from K. Sullivan (NIDR).

*Fibronectin Preparations.* Fibronectin was prepared from freshly drawn human serum by affinity chromatography on a denatured type I collagen–Sepharose column (Hopper et al., 1976; Engvall & Ruoslahti, 1977). Serum, after passage through this column, was found to be free of biologically active fibronectin as judged by its inability to promote the attachment of Chinese hamster ovary cells to collagen. The bound fibronectin was eluted with 1 M KBr, 0.05 M Tris-HCl, and 0.025 M 6-aminohexanoic acid, pH 5.3, then dialyzed against 0.02 M phosphate and 0.15 M NaCl, pH 7.4 [phosphate-buffered saline (PBS)], and concentrated by ultrafiltration. Cell-surface fibronectin was prepared from a urea extract of third-passage chick embryo fibroblasts (Yamada et al., 1977). In some cases, radioactive fibronectin was prepared from cells cultured for 24 h in medium containing [U-$^{14}$C]leucine (10 $\mu$Ci/mL). Fragments of plasma fibronectin were prepared by a brief digestion with chymotrypsin and separated by

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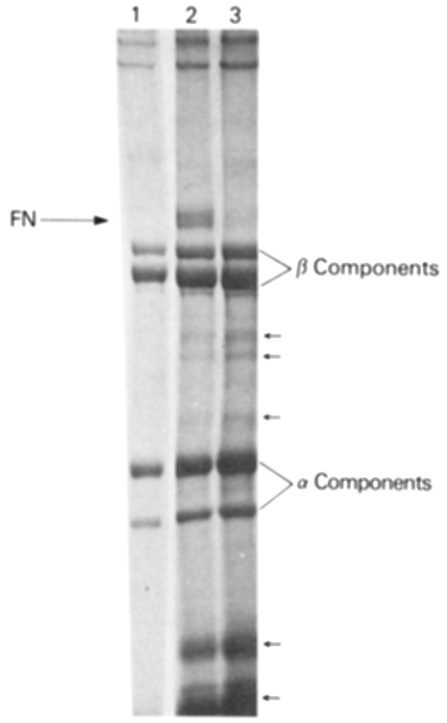


FIGURE 1: $NaDodSO_4$–polyacrylamide gel electrophoresis of serum proteins in rat tail collagen fibrils. Native collagen (200 μg) in PBS was mixed with 0.1 mL of serum in the cold in a final volume of 1 mL and then incubated for 1 h at 26 °C. The fibrils were collected by centrifugation, washed twice, redissolved in 0.5 N acetic acid, dialyzed against water, and lyophilized. Rat tail collagen gelled in the absence (lane 1) or presence of 0.1 mL of human serum (lane 2) or of 0.1 mL of human serum and 0.1 mg of α1(I)-CB7 (lane 3). The positions of plasma fibronectin, the α and β components of collagen, and the bound or trapped serum proteins are indicated.

collagen–Sepharose chromatography (Hahn & Yamada, 1979). The concentrations of fibronectin and its fragments were determined by the Lowry procedure (Lowry et al., 1951). Their purity was assessed by sodium dodecyl sulfate ($NaDodSO_4$) slab gel electrophoresis (Laemmli, 1970).

*Fibrillogenesis.* Fibrillogenesis of collagen was followed either by the increase in absorbance of the sample which occurred as collagen fibers formed (Gross & Kirk, 1958) or by sedimentation of the aggregated collagen. In these studies, 0.025 mL of rat tail tendon collagen in 0.5 M acetic acid was added in 0.5 mL of PBS. Various peptides and proteins dissolved in PBS were added to some samples, and the final volume was brought to 1.0 mL. In studies where fibril formation was to be followed turbidimetrically, the sample was placed in a jacketed flow cell maintained at 26 °C in a Cary spectrophotometer (Williams et al., 1978). The absorbance at 313 nm was monitored continuously.

In studies with labeled fibronectin, the samples were incubated in glass centrifuge tubes at either 26 or 37 °C. The amount of fibronectin used was always below that which would saturate all the sites on the collagen monomers. At various times, the samples were centrifuged at 10000 rpm for 10 min, and the pellet was resuspended in PBS and recentrifuged twice to remove associated, but soluble, material from the fibers. The fibers were dissolved in 0.5 M acetic acid, and aliquots were assayed for radioactivity by standard techniques. Other aliquots of the samples were electrophoresed under reducing conditions (Laemmli, 1970).

## Results

The binding of serum- and cell-derived fibronectin to different collagens was measured. In some studies, a solution of collagen prepared from rat tail tendon by acid extraction was incubated with serum and buffer at 4 °C, and the temperature was raised to 26 °C to initiate fibrillogenesis. Under these conditions, collagen readily aggregated into fibrils. The fibers that formed were sedimented, rinsed, and electrophoresed under reducing conditions (Figure 1). The presence of a closely spaced doublet band migrating at approximately 220000 daltons indicated that the fibers formed in the presence of serum contained fibronectin. No fibronectin sedimented in samples lacking collagen, indicating that a specific interaction occurred between collagen and fibronectin. Further evidence for a specific interaction between collagen and fibronectin was obtained by incubating collagen with serum in the presence of α1(I)-CB7, a cyanogen bromide generated peptide from the α1(I) chain containing the fibronectin binding site. Very low levels of fibronectin were bound to the collagen fibrils under these conditions (Figure 1, lane 3). A peptide, α1(I)-CB6, from a different region of the α1(I) chain was not as effective in reducing binding of plasma fibronectin (Table I). This peptide may have been contaminated with some α1(I)-CB7, or it may have some ability to bind to fibronectin (Kleinman et al., 1976; Dessau et al., 1978). These studies suggest that fibronectin binds to forming collagen fibers at the site identified previously as the fibronectin binding site in the α1(I) collagen chain (Dessau et al., 1978; Kleinman et al., 1978). Some other proteins from serum were also associated with the washed collagen fibers, but their relationship to fibronectin is not known. These bound proteins apparently do not bind to denatured collagen, since they are not seen in the material eluted from the gelatin–Sepharose columns and are not diminished when fibrils are formed in the presence of gelatin.

Table I: Factors Effecting the Binding of Fibronectin to Collagen[a]

| incubation mixture | fibronectin in precipitate (% of total) |
|---|---|
| fibronectin + native collagen | 80 |
| fibronectin[b] | 4 |
| fibronectin + denatured collagen[b] | 3 |
| fibronectin + native collagen + α1(I)-CB7 | 36 |
| fibronectin + native collagen + α1(I)-CB6 | 54 |
| fibronectin + native collagen + *Ascaris* collagen | 82 |
| fibronectin + native collagen + heparin | 45 |
| heated fibronectin (80 °C, 30 min) + native collagen | 60 |

[a] In each experiment, 5 μg of cell-surface fibronectin (5000 cpm) was used in a final volume of 1 mL. Native and denatured collagen were used at a final concentration of 400 μg/mL while the cyanogen bromide peptides, *Ascaris* collagen, and heparin were used at 200 μg/mL. In the presence of heparin, the amount of collagen precipitated was unchanged. All experiments were carried out in duplicate with results differing by less than 10%. [b] No precipitate was detected. At the concentration used, 5 μg/mL, cell-surface fibronectin is soluble at neutral pH.

Studies were also carried out on the binding of serum-derived fibronectin to collagen prepared from the skin of lathyritic rats. In solution, this collagen preparation is largely monodisperse and contains less of the higher aggregates (β and γ components) than the acid-extracted collagen. As in the previous experiments, serum-derived fibronectin bound to the washed fibers (Figure 2, lane 2). The binding of purified fibronectin, which was prepared from the cell surface of fibroblasts, to collagen was also studied. In the absence of collagen, very little fibronectin could be sedimented (Figure 3). The cell-surface fibronectin was bound to collagen fibers

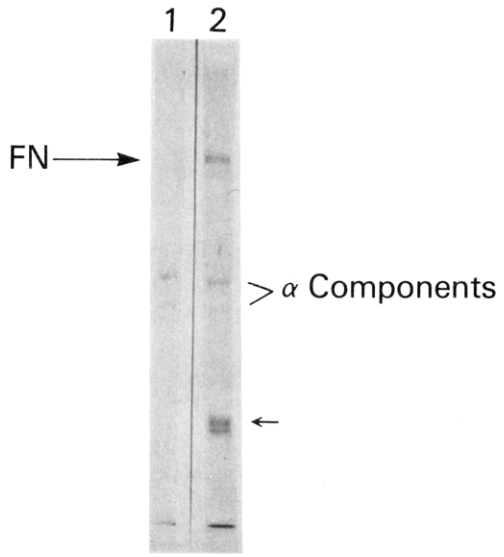


FIGURE 2: $NaDodSO_4$–polyacrylamide gel electrophoresis of serum proteins in lathyritic rat skin collagen fibrils. The fibrils were prepared and processed essentially as described in Figure 1. Lathyritic rat skin collagen was gelled in the absence (lane 1) or presence of 0.1 mL of serum (lane 2). The positions of plasma fibronectin, the α components of collagen, and the bound or trapped serum proteins are indicated.


FIGURE 3: Fluorograph of $NaDodSO_4$ gel electrophoresis of purified cell-surface fibronectin in rat tail collagen fibrils. All procedures were essentially as described in Figure 1 except 20000 cpm of $^{14}C$-labeled cell-surface fibronectin was present instead of serum. Fluorograph of $^{14}C$-labeled cell-surface fibronectin which was present in the pellet formed in the absence (lane 1) or presence of rat tail collagen (lane 2).

(Figure 3, lane 2), and its binding was also blocked by α1-(I)-CB7 (not shown).

Some factors affecting the binding of fibronectin to collagen fibers were assessed by a quantitative assay (Table I). Approximately 80% of the total fibronectin bound to the collagen fibers. Little fibronectin formed a precipitate in the absence of collagen or in the presence of denatured collagen which binds fibronectin. These studies indicate that it is the interaction of fibronectin with native collagen that is necessary for its presence in the insoluble complex. In this experiment, α1(I)-CB6 had some inhibitory activity, although α1(I)-CB7 was more potent. *Ascaris* collagen was without effect, as expected, since previous studies indicated that fibronectin does not bind to *Ascaris* collagen (H. K. Kleinman, unpublished experiments).

Table II: Effect of Fibronectin and Collagen on Time Required for Formation of Collagen Fibrils[a]

| incubation mixture | $t_{1/2}$ (min)[b] |
|---|---|
| control | 20 |
| albumin | 20 |
| cell-surface fibronectin | 30[c] |
| plasma fibronectin | 29[c] |
| cell-surface fibronectin heated 80 °C, 30 min | 26[c] |
| chymotryptic fragments: 40K | 32[c] |
| 160K | 20 |
| elastase fragments: 60K | 28[c] |
| 140K | 20 |
| α1(I)-CB7 (100 μg) | 22 |
| α1(I)-CB7 (100 μg) + fibronectin | 23 |

[a] In all cases, 0.2 μmol of fibronectin or fibronectin fragments was mixed with 200 μg of collagen in a final volume of 1.0 mL. Duplicates did not differ by more than 5%. Albumin was tested at 200 μg/mL. The fragments of plasma fibronectin prepared after neutrophil elastase digestion (McDonald & Kelley, 1980) were a gift from J. A. McDonald (Washington University, St. Louis, MO). [b] $t_{1/2}$ is the time required for half the maximal change in absorbance at 313 nm to be achieved. [c] Values significantly different from control.

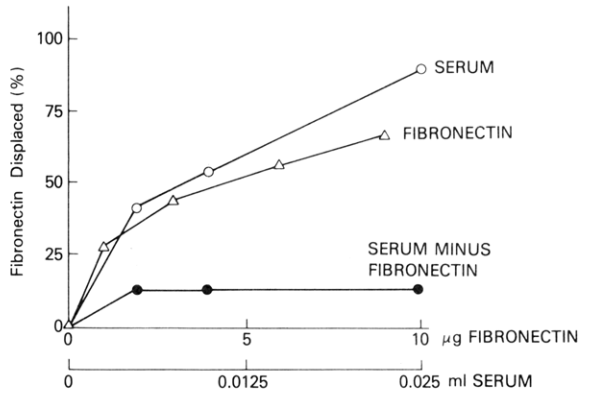


FIGURE 4: Effects of serum, fibronectin, and serum depleted of fibronectin on [$^{14}C$]fibronectin binding to native collagen fibrils. Increasing amounts of human serum, fibronectin prepared from the urea extracts of chick fibroblasts, or serum which had been passed over a collagen-Sepharose column to remove fibronectin were mixed with native collagen (150 μg) and 5 μg of $^{14}C$-labeled fibronectin prepared as described under Materials and Methods. After fibrils had formed, the precipitates were washed 2 times, redissolved in 1% acetic acid, and counted. Results of duplicate experiments differed by less than 10%.

Fibronectin heated to 80 °C for 30 min retained most of its ability to bind to collagen (Tables I and II) but did not mediate cell adhesion (data not shown). This indicates that the cell-binding domain is more labile than the collagen-binding domain. Heparin, which has been shown to enhance the binding of fibronectin to native collagen (Jilek & Hörmann, 1979; Johansson & Höök, 1980), had no effect in this system at concentrations below 50 μg/mL, but at higher concentrations it appeared to inhibit fibronectin binding.

We next determined the specificity of the binding of fibronectin to the forming fibrils in the presence of serum, fibronectin, and denatured collagen. Binding of 5 μg of $^{14}C$-labeled fibronectin to 150 μg of collagen was measured in the presence of known amounts of unlabeled fibronectin or serum (Figure 4). Approximately 5 μg of unlabeled fibronectin prevented 50% of the labeled fibronectin from binding to the collagen fibrils. Serum-containing fibronectin also reduced the amount of $^{14}C$-labeled (cell-surface) fibronectin bound in a concentration-dependent manner while serum from which the fibronectin had been removed had no effect. Denatured collagen, which does not sediment in the fibril assay, blocked

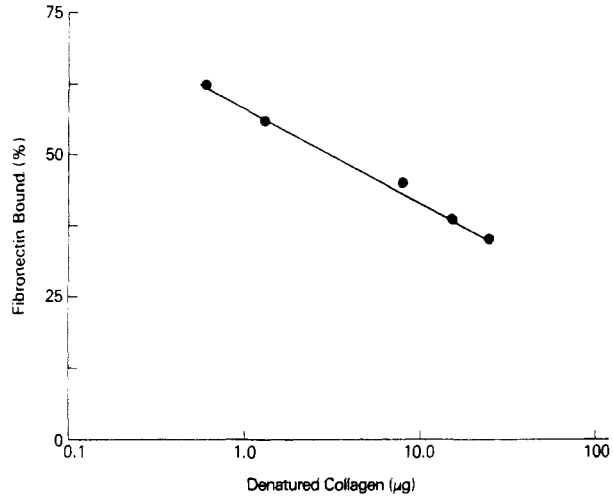


FIGURE 5: Effect of increasing amounts of denatured collagen on the binding of fibronectin to native collagen. Increasing amounts of boiled rat tail collagen were added to native rat tail collagen (150 μg) and 5 μg of [$^{14}$C]fibronectin, as described in Figure 1. After 1 h, the bound fibronectin was determined as described in Figure 2.

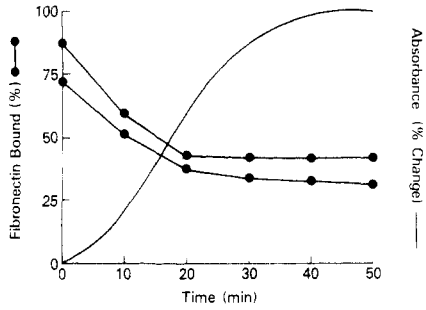


FIGURE 6: Binding of fibronectin to native collagen at various stages during fibril formation. Fibril formation in the absence of fibronectin was monitored by the absorbance at 313 nm. Collagen was allowed to form fibrils as described in Figure 1. At the times indicated, 5 μg of $^{14}$C-labeled fibronectin was added to the mixture and incubated with the collagen for exactly 1 h in all cases. The fibrils were then collected and assayed for bound fibronectin as described in Figure 2. Two separate experiments are shown. Each point represents the average of duplicates which did not differ by more than 10%.

the binding of fibronectin to the forming fibrils in a concentration-dependent manner (Figure 5). Thus, the interaction of fibronectin with native collagen is specific since it it blocked only by denatured collagen and not by a mixture of serum proteins (from which the fibronectin has been removed).

The ability of fibronectin to bind to collagen at various times during fibril formation was determined (Figure 6). A decrease in fibronectin binding occurred as the collagen fibers formed. This is presumably due to inaccessibility of fibronectin binding sites on collagen molecules located in internal positions in the collagen fibers.

Since fibronectin binds to native collagen, we considered the possibility that it could affect the rate of fibril formation. Fibronectin from either serum (not shown) or the cell surface (Figure 7) inhibited the rate of fibril formation in a concentration-dependent manner but did not alter the final extent of fibril formation, based on the absorbance at 313 nm. In the presence of α1(I)-CB7, fibronectin did not delay fibrillogenesis (Table II). This suggests that fibronectin delays fibrillogenesis because it binds to the collagen molecule. This effect is specific for fibronectin since albumin, chondronectin (Hewitt et al., 1980), and laminin (Timpl et al., 1979) did not affect fibrillogenesis (data not shown). In addition, specific fragments of fibronectin were tested for their effects on fibril

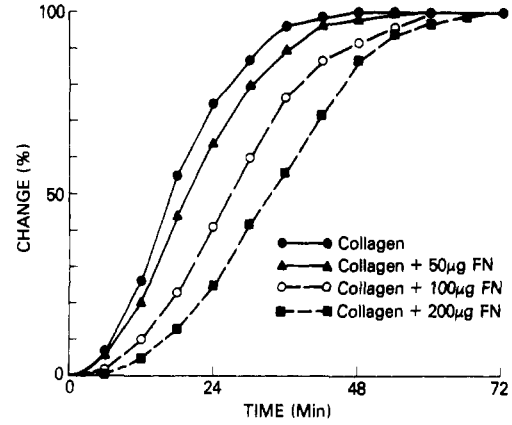


FIGURE 7: Effect of fibronectin on the rate of fibril formation. Increasing amounts of fibronectin (FN) prepared from urea extracts of chick fibroblasts were added to native collagen (200 μg) as described in Figure 1. The temperature was raised to 26 °C, and the fibril precipitation was monitored by the absorbance at 313 nm. The final extent of fibril formation as monitored by absorbance was unchanged.

formation (Table II). Peptides containing the cell-binding portion of the molecule (140 000 and 160 000 daltons) were without effect while peptides containing the collagen-binding region (40 000 and 60 000 daltons) delayed fibrillogenesis in a concentration-dependent manner and were as active on a molar basis as the parent molecule. Fibronectin which had been heated to 80 °C for 30 min was unable to promote cell attachment (not shown) but still bound to collagen (Table I) and delayed fibrillogenesis (Table II) although with reduced activity. Thus, a specific portion of fibronectin known to bind to collagen is responsible for delaying fibrillogenesis.

## Discussion

Fibronectin purified from both serum and cell surfaces binds to native collagen. When collagen molecules aggregate in the presence of serum, in which fibronectin represents about 1% of the protein (Mosesson & Umfleet, 1970), fibronectin and only a few other proteins are bound to the collagen. The binding of fibronectin is not due to simple ionic bonds since it is blocked by α1(I)-CB7 but not to the same extent or at all by other collagenous molecules, such as α1(I)-CB6 and *Ascaris* collagen. Given the relatively hydrophobic nature of the putative binding site, uncoiling of the helix and binding of fibronectin would be favored at physiological temperatures. Although fibronectin binds better to forming fibers than to performed fibers, perhaps for steric reasons, it is also possible that the fibers are partially altered by fibronectin. The simplest explanation for the data is that the binding site for fibronectin is the same in both native and denatured collagen.

Fibronectin has been shown to contain various domains which interact specifically with collagen, with the surface of cells, with transglutaminase, and with heparin and hyaluronic acid (Hahn & Yamada, 1979; Balian et al., 1979; Ruoslahti et al., 1979; Sekiguchi & Hakamori, 1980; McDonald & Kelley, 1980; Yamada et al., 1980). These domains are thought to be globular (Alexander et al., 1978) and interconnected by flexible, nonglobular sequences. A specific domain in the fibronectin molecule has been isolated which binds to collagen but is unable to promote cell adhesion (Hahn & Yamada, 1979; Ruoslahti & Hayman, 1979). The collagen-binding region can be generated by a variety of proteases, including chymotrypsin, trypsin, thermolysin, elastase, and subtilisin, but is resistant to further degradation by these enzymes. This domain is also relatively heat stable. Fibronectin loses its ability to promote cell adhesion when heated

to 60 °C for 30 min, but even after it is heated to 80 °C for 30 min it can still bind to collagen and delay fibrillogenesis, although to a somewhat reduced extent. Thus, in a wound or inflamed tissue where fibronectin might be partially degraded, its collagen binding domain might remain functional.

It has been known for many years that collagen self-assembles into fibrils (Wood, 1964). Most in vitro studies have been unable to reproduce the homogeneity in size of native collagen fibrils produced in vivo perhaps due to alterations in the collagen molecule during purification or to the absence of necessary cofactors. It is known, for example, that the nonhelical extensions of collagen are required for the early conformational changes necessary for the assembly of fibrils (Comper & Veis, 1977; Gelman et al., 1979; Helseth et al., 1979; Silver & Trelstad, 1979). In addition, certain proteoglycans and glycosaminoglycans bind to collagen and influence the rate of fibril formation and the final size of the fibrils (Wood, 1960; Keech, 1961; Mathews & Decker, 1968; Toole & Lowther, 1968; Lee-Own & Anderson, 1975; Oegema et al., 1975; Greenwald et al., 1975; Toole, 1976) but not the extent of fibril formation. Various investigators have observed different effects of proteoglycans or glycosaminoglycan on collagen fibrillogenesis, probably due to the source of the reacting molecules or to the assay conditions. However, early studies showed that molecules such as heparin which delay fibrillogenesis yield thick fibrils while those such as chondroitin sulfate that increase the rate of fibrillogenesis result in thin fibrils (Wood, 1960; Keech, 1961). Fibronectin clearly binds to native collagen fibrils as demonstrated both in this and in previous studies (Furcht et al., 1980; Johansson & Höök, 1980). Since fibronectin delays fibrillogenesis, it could possibly regulate the size of the collagen fibrils in vivo. Furthermore, fibronectin interacts with proteoglycans (Stathakis & Mosesson, 1977; Jilek & Hörmann, 1979; Yamada et al., 1980; Ruoslahti & Engvall, 1980), and its binding to native collagen is enhanced by heparin (Johansson & Höök, 1980). Thus, it is possible that fibronectin interacting with proteoglycans and/or other molecules controls fibril formation in vivo.

The ability of fibronectin to bind to native collagen is physiologically significant in that the resulting matrix can support cell adhesion and provide tissue integrity, and, in wound repair, it can provide a scaffolding for platelet adhesion and aggregation and other repair reactions. Since fibronectin also influences the rate of fibrillogenesis, it may influence the length and width of collagen fibers.

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# Heavy-Chain Mutants Derived from $\gamma_{2b}$ Mouse Myeloma: Characterization of Heavy-Chain Messenger Ribonucleic Acid, Proteins, and Secretion in Deletion Mutants and Messenger Ribonucleic Acid in $\gamma_{2a}$ Mutant Progeny†

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ABSTRACT: Mouse myeloma mutants isolated from cell line 45.6 ($\gamma_{2b}$) producing structurally altered immunoglobulin heavy (H) chains have been characterized. The mutant 10-1 synthesizes an H chain of 47 000 daltons containing a $CH_1$ deletion; two mutants, G251 and I17, derived from 10-1 synthesize H chains of 40 000 and 35 000 daltons, respectively. The messenger ribonucleic acids (mRNAs) in these mutants have been shown to be smaller in molecular weight than mRNAs produced in 45.6 cells and lack a portion, but not all, of the $CH_1$ domain. The H chains of G251 and I17 no longer express IgG subclass-specific determinants, are not secreted, and are structurally altered in the carboxy-terminal portion of the molecule. In vitro the mRNAs of the mutants code for the synthesis of a polypeptide precursor characteristic of secreted proteins; the shortened proteins are apparently glycosylated intracellularly. Somatic cell hybrids between a structurally altered nonsecretor and a drug-marked wild-type myeloma cell secrete only the wild-type protein. Reversion to secretion for G251 or I17 is accompanied by a change in the amino acid composition of the H chain such that $\gamma_{2a}$ subclass-specific determinants are expressed. Therefore, the primary structure of the H chain is an important factor in determining secretion. The $\gamma_{2a}$-secreted chains from G251 and I17 fall into two classes: (1) those synthesizing proteins of ~47 000 daltons producing H-chain mRNAs of ~1.66 kilobases that are deleted for a portion, but not all, of $CH_1$; (2) those synthesizing $\gamma_{2a}$ proteins of ~55 000 daltons that are encoded in mRNAs of apparently wild-type size and that have regained $CH_1$ sequences. The molecular explanations for the production of these alterations is discussed.

Cultured myeloma cells provide a suitable system in which to study the biosynthesis of proteins, mutational events, and gene rearrangements in eukaryotic cells. The myeloma cells are derived from a plasma cell tumor and usually synthesize a single immunoglobulin (Ig) molecule. Both the wild-type and mutant genes and gene products of such cell lines are available for detailed characterization.

The multiple domain structure of the Ig heavy (H) chain protein is reflected in the deoxyribonucleic acid (DNA)[1] encoding it. For example, the active IgG ($\gamma_{2b}$) heavy-chain gene of the mouse myeloma has intervening sequences separating the V, $CH_1$, hinge, $CH_2$, and $CH_3$ domains (Maki et al., 1980). In both heavy and light chains, the variable (V) region of the expressed gene is constructed from the rearrangement of discrete DNA sequences (Sakano et al., 1979; Max et al., 1979; Early et al., 1980; Schilling et al., 1980). Each antibody-producing cell appears to be committed to the production of a defined $V_L$, $C_L$, and $V_H$ for its entire differentiation from B lymphocyte to plasma cell. However, many different heavy-chain constant region genes may be expressed with one $V_H$. Initially, a B cell expresses $C_\mu$ (Raff, 1976); these immature B cells may further develop to produce another class of heavy chain. This class switch seems to be accomplished by a DNA rearrangement (Davis et al., 1980) presumably associated with the deletion of preceding heavy-chain constant region genes (Honjo & Kataoka, 1978; Cory & Adams, 1980; Rabbits et al., 1980; Coleclough et al., 1980). In addition to normal lymphocytes, myeloma cells in culture also have been shown to switch the subclass of IgG being produced

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[1] Abbreviations used: DNA, deoxyribonucleic acid; cDNA, complementary DNA; RNA, ribonucleic acid; mRNA, messenger RNA; hnRNA, heterogeneous nuclear RNA; Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol; $NaDodSO_4$, sodium dodecyl sulfate; poly(A), poly(adenylic acid); SSC, standard saline citrate; EDTA, ethylenediaminetetraacetic acid; VTL, valine, threonine, and leucine.

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